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A high-amylopectin diet caused hepatic steatosis associated with more lipogenic enzymes and increased serum insulin concentration

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Abstract

Starch is the major energy source for monogastric mammals and humans. The present study was conducted to evaluate the liver metabolic responses of weaned pigs fed with different dietary starches. A total of sixteen weaned pigs were fed with two experimental diets containing either cassava starch (CS, 80 % amylopectin and 20 % amylose) or maize starch (70 % amylopectin and 30 % amylose). The present results showed that the growth performance was not affected by different dietary starches ($P>0{\cdot}05$). However, ingestion of CS not only increased the lipid content in liver tissues, but also elevated the concentrations of serum cholesterol and insulin ($P<0{\cdot}05$). The metabolic responses induced by CS were associated with more lipogenic enzymes such as fatty acid synthase and 3-hydroxy-3-methyl-glutaryl-CoA reductase in liver ($P<0{\cdot}05$). Real-time PCR quantification for lipid metabolic genes indicated that ingestion of CS not only up-regulated the expression of these lipogenic genes, but also decreased the expression of lipolytic genes. These results suggested that the metabolic responses of weaned pigs fed with different dietary starches may vary widely depending on their composition, and ingestion of starches that are high in amylopectin may produce a stronger insulinaemic response and lead to an up-regulation of lipogenesis in the liver.

Key words: Starch nutrition: Energy metabolism: Lipid: Liver: Weaned pigs

Dysfunctional energy metabolism underlies the development of obesity and obesity-related complications such as hepatic steatosis, diabetes and heart disease[1]. It is a well-known fact that carbohydrate is the most prevalent source of energy for monogastric mammals and humans. Carbohydrates not only provide the substrate for the Krebs cycle, but also act as a regulator for lipid metabolism[2,3]. Upon consumption of excess carbohydrate, digestion yields simple sugars that are converted to pyruvate (glycolysis), which is either oxidised to provide energy or channelled into pathways for synthesis of fatty acids (lipogenesis) when energy is available[3]. The coordinated regulation of these metabolic processes allows the efficient utilisation of dietary carbohydrates, and key enzymes involved in carbohydrate metabolism are tightly regulated by hormones and dietary nutrients[4].

Previous studies have indicated that in monogastric mammals the metabolic responses induced by different dietary carbohydrates are widely variable[5,6]. In humans, isoenergetic replacement of dietary starch with sucrose, in the short term, resulted in elevated plasma TAG and cholesterol concentrations[6,7] and impaired glucose tolerance[8]. Similar adverse effects of high sucrose consumption have been reported for non-human primates and a number of laboratory animals[2,9]. Starch, acting as the main energy source of the daily diet, is the most prevalent carbohydrate consumed by monogastric mammals. However, the metabolic responses induced by different dietary starches may vary widely depending upon their sources and polymer structures[10,11]. For instance, starches that are high in amylopectin are easily digested, which may lead to a rapid increase in blood glucose and insulin concentration, whereas starches with more amylose may result in moderate glycaemic and insulinaemic responses[12–14]. Although these metabolic responses were previously studied in both humans and other monogastric mammals, still less is known about the mechanisms behind these responses. More importantly, few studies have investigated the relationship between hepatic lipid metabolism and starch composition. Therefore, the aim of the present study was to evaluate the metabolic responses of weaned pigs fed with different dietary starches, and mechanisms behind these responses were investigated on a molecular basis.

Abbreviations: CS, cassava starch; GH, growth hormone; MS, maize starch.

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Materials and methods

Starches

Purified cassava starch (CS, 80% amylopectin and 20% amylose) and maize starch (MS, 70% amylopectin and 30% amylose) were purchased from Chengdu food market (Chengdu, Sichuan, China).

Animals and diets

The experimental protocols used in the present study were approved by Sichuan Agricultural University Institutional Animal Care and Use Committee. Sixteen weaned pigs (Duroc × Landrace × Yorkshire) with an average initial body weight of 7·37 (SEM 0·25) kg were selected and randomly allotted to two dietary treatments with equal numbers of males and females in each group. The experimental diet was formulated on the basis of nutrient requirements established by the National Research Council (1998) for 5–10 kg pigs[15]. Either CS or MS was used as the sole dietary energy source. There were no discrepancies for other nutrient components. Dietary amino acids were supplied by dehulled soyabean meal, extruded soyabean and fishmeal, and vitamin and minerals were supplied by vitamin and mineral supplements (Table 1). Synthetic DL-methionine was added to the diets to meet minimal methionine–cystine requirements.

Animal housing and tissue sampling

The pigs were housed individually in metabolism cages (0·7 × 1·5 m) with woven wire flooring in an environmentally controlled room (22–24°C) and were given *ad libitum* access to water through a water nipple. They were hand-fed four times/d (08.00, 12.00, 16.00 and 20.00 hours) in bowl feeders to make sure fresh feed was available, and were allowed a 7 d adjustment to the experimental diets. The diet adjustment period was followed by a 21 d experimental period. Weights and feed consumption of the pigs were determined daily throughout the trial. The blood samples were collected by venepuncture at 07.00 hours on day 14. At the end of the trial, pigs were euthanised with an intravenous injection of pentobarbital sodium (50 mg/kg body weight) and the liver samples were collected, weighted and stored at −80°C.

Biochemical analysis

The liver and serum lipids were extracted and purified[16]. TAG and cholesterol levels were measured using the method described by Hercberg *et al.*[17]. Insulin, growth hormone (GH) and glucagon levels were measured using electrochemiluminescence immunoassays (Roche Diagnostics, Meylan, France). All the assay kits were purchased from Tosoh Corporation (Kyoto, Japan). The activities of liver glucose-6-phosphate dehydrogenase, fatty acid synthase, acyl-CoA oxidase and 3-hydroxy-3-methylglutaryl-CoA reductase were assayed according to methods described by Ide *et al.*[18].

Table 1. Ingredient and chemical composition of experimental diets (as fed-basis)

	Chemical composition	
Ingredients (% of diet)	CS	MS
CS	54·50	
MS		54·50
Dehulled soyabean meal	2·00	2·00
Extruded soyabean	10·00	10·00
Soya protein concentrate	17·83	17·83
Whey powder	7·30	7·30
Fishmeal	6·00	6·00
$CaHPO_4$	0·70	0·70
$CaCO_3$	0·55	0·55
Salt	0·15	0·15
Choline chloride (50%)	0·10	0·10
L-Lysine	0·00	0·00
Methionine	0·17	0·17
Threonine	0·01	0·01
Cr_2O_3	0·40	0·40
Trace mineral premix*	0·20	0·20
Vitamin premix†	0·04	0·04
Additives	0·05	0·05
Total	100·00	100·00
Dietary energy (MJ/kg)	14·50	14·50
Crude protein (%)	19·99	19·99
Ca (%)	0·80	0·80
Available P (%)	0·41	0·41
L-Lysine (%)	1·29	1·29
Methionine + cystiene (%)	0·74	0·74
Threonine (%)	0·81	0·81
Tryptophan (%)	0·25	0·25
Arginine (%)	1·49	1·49
Na (%)	0·20	0·20
Crude fibre (%)	1·37	1·37
Crude fat (%)	2·60	2·60
Ash (%)	3·21	3·21

CS, cassava starch; MS, maize starch.

* Supplied (per kg diet): Fe as $FeSO_4·7H_2O$, 100 mg; Mn as $MnSO_4·7H_2O$, 40 mg; Zn as ZnO, 80 mg; Cu as $CuSO_4·5H_2O$, 10 mg; Se as $NaSeO_3$, 0·3 mg and I as KI, 0·3 mg.

† Supplied (per kg diet): 5·7 mg vitamin A, 36·7 mg vitamin E, 0·01 mg vitamin D, 1·1 mg vitamin K (menadione dimethylpyrimidinoe bisulfate), 5 mg vitamin B_1, 15 mg riboflavin, 25 mg niacin, 30 mg D-pantothenic acid and 0·05 mg vitamin B_{12}.

RNA extraction

Total RNA was isolated from liver using TRIzol (Invitrogen, Carlsbad, CA, USA) and further purified by RNeasy Mini Kit (Qiagen, Valencia, CA, USA). All the procedures were carried out as per the manufacturer's protocol. The concentration of RNA was determined using spectrophotometry based on absorbance at 260 nm and integrity was monitored using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

Real-time RT-PCR

Real-time PCR primers were designed (Takara, Dalian, Liaoning, China) to assay six genes related to lipid metabolism (Table 2). *β-Actin* was used as the reference gene. Briefly, 500 ng RNA was reverse transcribed using high-capacity cDNA Reverse Transcription Kit (PN 4368814; Invitrogen) for each pig. Real-time RT-PCR for six target genes and the housekeeping gene were performed using Applied Biosystems (Foster City, CA, USA) Power SYBR Green PCR Master Mix

Table 2. Primer sequences of genes selected for analysis by real-time RT-PCR

Gene	Accession no.	Forward primer	Reverse primer	Temp (°C)
hmgr	NM_001122988	GAGTGGTCCCACAAATGAAG	CACGGTCCCGATCTCTATG	60·5
acox1	AK232470	TGACGGGAATGTGTATGAAA	CAGGTGCTTGTGGTAAGA	59·1
fasn	NM_001099930	GTGTGAGCAGTTCTGATG	AGCCTATCATGCTGTAGC	59·5
cpt1a	NM_001129805	ACAAGCCATAGTCTTAACGAAA	GCCAGTCCAGGATAACAAA	59·5
pparα	AK232864	CGTATCCTGCGTATGAAG	GTGTGAGCCTAAGAAGTT	58·5
dgat	NM_214051	ACCTACCGCGATCTCTAC	AGCTGGATGAGGAACAGCAT	59·6
β-Actin	AY550069	TCTGGCACCACACCTTCT	TGATCTGGGTCATCTTCTCAC	56·5

Temp, temperature.

in a Bio-Rad iCycler with minor modifications (Bio-Rad, Hercules, CA, USA). Fluorescein was added at a final concentration of 10 nM as the reference dye. Cycling conditions were as follows: 95°C for 5 min, forty-five cycles of 95°C for 30 s, appropriate annealing temperature (Table 2) for 30 s, 72°C for 30 s, followed by 72°C for 5 min, 95°C for 1 min, 55°C for 1 min, followed by a melt curve analysis of eighty cycles of 10 s at 55°C with a 0·5°C increase every cycle.

Statistical analysis

Gene expression data from replicate samples were averaged and analysed using the Pfaffl[19] method to measure the difference between the cassava and maize cycle threshold values. Growth performance, metabolic enzymes and serum data were analysed by SPSS 13.0 (SPSS, Inc., Chicago, IL, USA). Determination of statistical significance was carried out by independent-sample *t* test. Data were expressed as means with their standard errors. Differences with $P<0·05$ were considered to be significant.

Results

Growth performance

The growth parameters are reported in Table 3. There were no significant differences ($P>0·05$) in either average daily body weight gain or feed intake between the CS and MS groups during the 21 d experimental period; weight gains were 378·9 (SEM 21·3) and 386·7 (SEM 28·2) g/d in the CS and MS groups respectively. The daily feed intakes of the CS and MS groups were 492·7 (SEM 29·1) and 509·4 (SEM 35·6) g/d respectively.

Table 3. Effects of different dietary starches on growth performance, metabolic hormones and hepatic lipid concentrations in weaned pigs

(Mean values with their standard errors)

	CS		MS	
	Mean	SEM	Mean	SEM
Average daily gain (g/d)	378·9	21·3	386·7	28·2
Average daily intake (g/d)	492·7	29·1	509·4	35·6
Serum growth hormone (ng/ml)	1·03	0·04	0·92	0·03
Serum insulin (pmol/l)	72·42*	5·93	56·24	5·17
Serum glucagon (pg/ml)	31·33	6·12	26·51	5·74
Liver total fat (g/100 g wet tissue)	8·91*	0·62	5·38	0·33
Liver TAG (μmol/g)	69·12†	7·11	58·34	6·32
Liver cholesterol (μmol/g)	3·42**	0·25	2·78	0·19

CS, cassava starch; MS, maize starch.

Mean value was significantly different from that of the MS group: * $P<0·05$, ** $P<0·01$.

† Mean value tended to be significantly different from that of the MS group ($P<0·1$).

Serum metabolites and hormones

No significant difference in serum glucose concentration was observed between the two groups (Fig. 1). However, ingestion of CS acutely increased the serum cholesterol concentration (2·04 (SEM 0·13) *v.* 1·54 (SEM 0·10) mmol/l, $P<0·05$). In addition, the serum TAG concentration increased by 14·9% in the CS group (Fig. 1). There were no significant differences in serum GH and glucagon concentrations between the two groups ($P>0·05$). In contrast, ingestion of CS significantly elevated the serum insulin concentration (Table 3).

Lipid content and metabolic enzymes in liver

Ingestion of CS significantly elevated the liver total fat and cholesterol concentration ($P<0·05$). In addition, the TAG concentration increased by 18·5% in this group (Table 3). The activities of several critical enzymes involved in lipid metabolism were measured (Fig. 2). No significant difference was observed for the activity of glucose-6-phosphate dehydrogenase between the two groups ($P>0·05$). However, ingestion of CS significantly elevated the fatty acid synthase and 3-hydroxy-3-methyl-glutaryl-CoA reductase activities in the liver ($P<0·05$). The activity of acyl-CoA oxidase was lower in the CS group than that in the MS group ($P<0·05$).

Hepatic gene expression

Quantitative real-time RT-PCR assays were designed for six genes expressed in liver. The genes were selected based on their involvement in lipid metabolism or their importance as components of the metabolic process. The present results indicated that ingestion of CS significantly elevated the transcription of lipogenic genes such as fatty acid synthase and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (Fig. 3). However, the expression of lipolytic genes such as acyl-CoA oxidase 1 (*acox1*) and *pparα* decreased 1·18- and 1·24-fold respectively ($P<0·05$). No significant differences were observed for diacylglycerol acyltransferase and carnitine palmitoyltransferase 1A transcription between the two groups ($P>0·05$).

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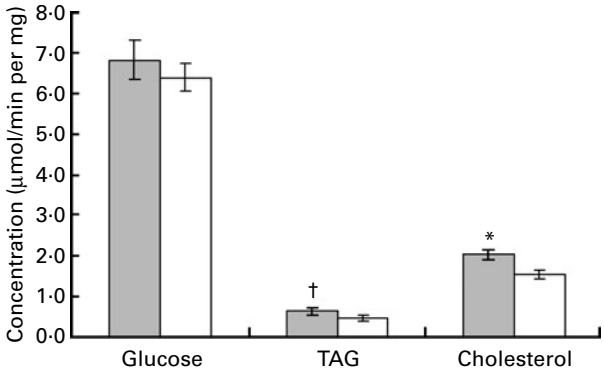

Fig. 1. Effect of different dietary starches on serum glucose and lipid concentrations. Mean values were significantly different: *$P<0·05$, †$P<0·10$. ▨, Cassava starch (CS); □, maize starch (MS).

Discussion

Starch is the most important energy source for monogastric mammals. However, the digestibilities of starches from different sources are widely variable[10,11,13]. Starches with a high amount of amylose are difficult to hydrolyse, whereas fully gelatinised amylopectin is easily digested, which can serve as a source of rapidly digestible starch and cause a stronger glycaemic and insulinaemic responses[10–13]. In the present study, the growth performance of weaned pigs was not affected by different dietary starches. However, ingestion of CS significantly elevated the serum cholesterol (32·4 %) and insulin (28·8 %) concentrations ($P<0·05$). Previous studies have indicated that ingestion of carbohydrate results in elevated blood glucose, which rapidly triggers insulin release from β-cells of the endocrine pancreas[11,20]. Unexpectedly, no significant difference was observed for serum glucose concentration between the two groups. This should be attributed, in part, to the elevated serum insulin concentration in the CS group (Table 3) and the time point for blood collection (blood samples were collected before the first meal in the morning). According to a previous report, the average retention time of starch digestion in small intestine is about 4 h, postprandial

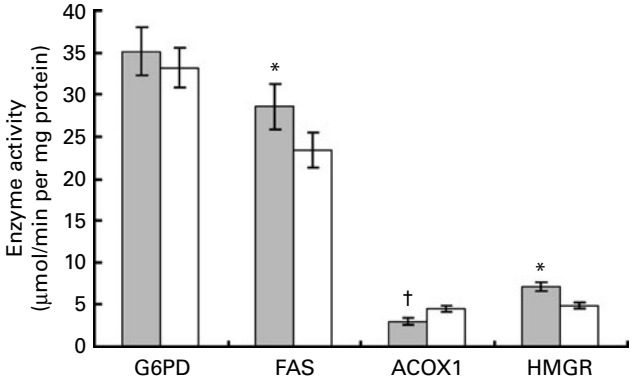

Fig. 2. Effect of different dietary starches on the activities of lipid metabolic enzymes. Mean values were significantly different: *$P<0·05$, †$P<0·10$. ▨, cassava starch (CS); □, maize starch (MS). G6PD, glucose-6-phosphate dehydrogenase; FAS, fatty acid synthase; ACOX1, acyl-CoA oxidase 1; HMGR, HMG-CoA reductase.

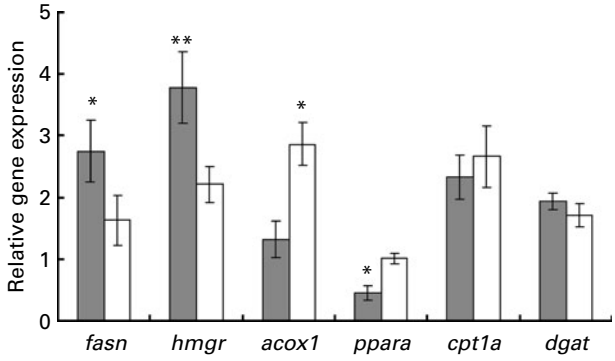

Fig. 3. Effect of different dietary starches on hepatic gene expression. The relative expression was calculated as the ratio of target gene to internal reference gene. Mean values were significantly different: *$P<0·05$; **$P<0·01$. ▨, Cassava starch; □, maize starch; *fasn*, fatty acid synthase; *hmgr*, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; *acox1*, acyl-CoA oxidase 1; *cpt1a*, carnitine palmitoyltransferase 1A; *dgat*, diacylglycerol acyltransferase.

circulated glucose as well as other metabolites may change periodically[10]. The present results, however, agree well with previous findings showing that ingestion of a quickly digested carbohydrate significantly elevated the plasma TAG and insulin concentrations in rats[21]. Furthermore, a stronger insulinaemic response was previously observed both in humans[22] and other monogastric animals[23–25] after ingestion of starches high in amylopectin. We also measured the concentrations of serum GH and glucagon. GH is a protein-based polypeptide hormone capable of stimulating growth and cell reproduction and regeneration in humans and other animals, whereas both insulin and glucagon are important hormones involved in carbohydrate metabolism. In the present study, no significant differences were observed for serum GH and glucagon concentration between the two groups.

The liver is the central player in whole-body energy homoeostasis. We have found that ingestion of CS deposited more lipids in the liver (Table 3). Similar results were observed in a rat model, which showed that a diet high in rapidly absorbed carbohydrate causes hepatic steatosis[21]. In the present study, the liver total fat concentration exceeded 8 % in the CS group, which indicated a moderate fatty liver. The elevated liver fat concentration in the CS group might result from the elevated insulin concentration since it has long been looked as one of the most important hormones to activate the transcription of lipogenic enzymes[4,26]. This hypothesis was also verified by the measurements of the enzyme activities produced in liver tissues (Fig. 2). Fatty acid synthase plays a key role in fatty acid synthesis, whereas 3-hydroxy-3-methylglutaryl-CoA reductase is the rate-controlling enzyme of the mevalonate pathway that produces cholesterol and other isoprenoids[27,28]. The present results indicated that the activities of both enzymes were elevated in the CS group ($P<0·05$). However, ingestion of CS significantly decreased the activity of acyl-CoA oxidase – a key enzyme involved in the fatty acid β-oxidation pathway[29,30].

To explore the mechanisms behind these metabolic responses, we analysed the transcription levels of six important genes involved in lipid metabolism. Ingestion of CS

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significantly activated the transcription of lipogenic genes – fatty acid synthase and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (Fig. 3). The acox1-encoded protein is the first enzyme of the fatty acid β-oxidation pathway, and defects in this gene result in accumulation of very long-chain fatty acids in the body[31]. We found that the transcription of acox1 was down-regulated in the CS group (Fig. 3). The real-time PCR results agree well with the enzyme activities produced in the liver (Fig. 2). The PPARα-encoded protein is an important transcriptional factor involved in the regulation of energy metabolism[32]. In the present study, the transcription of PPARα was down-regulated in the CS group (Fig. 3). PPARα belongs to the PPAR subfamily of nuclear receptor and facilitates energy combustion by activating the transcription of catabolic genes (i.e. *FABP3*, *CYP4A1* and *ADIPO*; Fatty acid binding protein 3, cytochrome P450 and adiponectin, respectively) involved in lipid catabolism[33]. The role of PPAR in hepatic steatosis has been fully investigated[34]. The present results indicated that the ingestion of starches with more amylopectin may increase the incidence of hepatic steatosis by down-regulation of the PPARα signalling pathway. Therefore, PPARα can be a valuable target for nutritional intervention during the process of steatosis, and starches with less amylopectin may help prevent or treat obesity and fatty liver in humans.

In summary, the present results suggested that the metabolic responses of weaned pigs fed with different dietary starches may vary widely depending on their composition, and ingestion of starches that are high in amylopectin not only induces a stronger insulinaemic response, but also leads to an up-regulation of lipogenesis and steroidogenesis in the liver.

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J. H. and B. Y. participated in the experimental design, carried out the molecular and biochemical experiments, participated in data interpretation and helped draft the manuscript. B. Y. conceived the study. D. C. and K. Z. directly supervised the project, participated in its experimental design and data interpretation. J. H. was also responsible for writing the manuscript. The authors state that there are no conflicts of interest in this field. This work was supported by Youth Fund Project of Sichuan Ministry of Education (grant no. 00924200) and Program for Changjiang Scholars and Innovative Research Team in University (grant no. IRTO555-5), China Ministry of Education.

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